REMARKS

The Specification has been amended to include reference to the issued U.S. Patent 6,780,602.

Applicants provide the following remarks to address the Examiner's rejection of the claims under 35 USC § 102(b) and 103(a).

The present application describes a method for the identification of a biological analyte with a ligand conjugated to a label, separating the bound analyte from excess marker-conjugated ligands in solution with another ligand conjugated to a surface with a photostable linker of disclosed length, and detecting the presence of the bound analyte through the signal from the marker. Claim 1 of Piran discloses non-competitive immunoassays (column 4, lines 22-25) for the detection of analytes which can be conducted in a single container (claim 1). Examples of the use of their invention to accomplish these assays are provided in Figures 1 and 3 of the Piran patent. In both these applications of their invention, the removal of excess label-conjugated binders (antibodies) is predicated and is accomplished with the use of "an insoluble material which is attached by a reversible bridge to an analyte derivative or an analyte mimic" (claim 1). The removal of 'analyte-free' labeled binder is what makes this invention a non-competitive assay, and exhibits the difference between removing the non-reacted labeled antibody (the method of Piran) and the present application (in which the analyte is removed from the solution). The necessity for the reversible bridge in conjunction with a wash solution to dissociate the labile bond between analyte mimic/derivative to reduce background signal is also required (column 5, lines 39-44). Though the method disclosed in the present application differs from that taught by Piran, after careful

assessment of the examiner's comments, claim 1 has been amended to limit the declared invention to fully incorporate what is novel in the current application without traversing the invention of Piran, et al.

As pointed out by the examiner, Piran discloses the use of labels that are fluorescent, luminescent and radioactive. However, Piran teaches that these labels are known to those skilled in the art (column 7, lines 15-18) citing Howanitz, et al. (column 7, lines 24-28) which includes these labels. Claims 10-12 of the current application teach the use of fluorescent, luminescent and radioactive markers (labels), but limit their use to the specific application as taught in the amended claim 1.

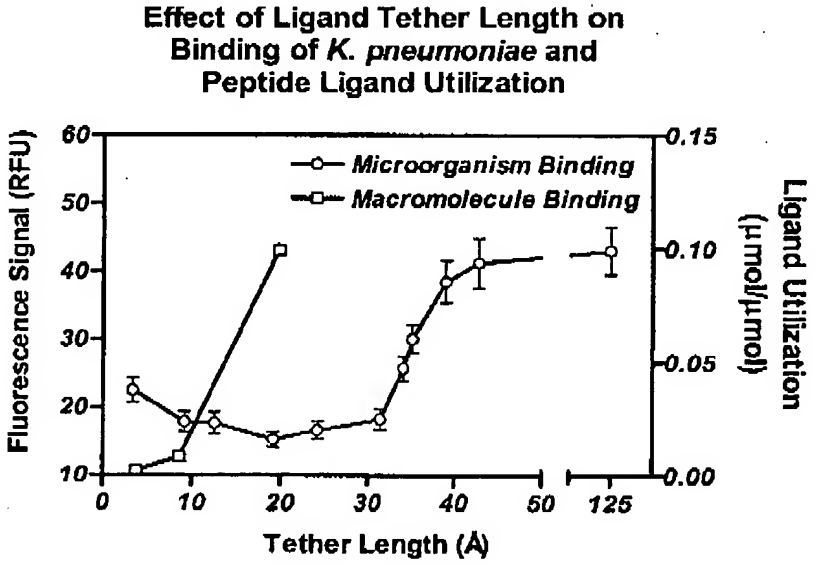
Piran discloses non-competitive immunoassays for the detection of analytes which can be conducted in a single container (Figures 1 and 3, claim 1) or multiple containers (Figures 2, 4-7). (Piran cites Jackson and Ekins (T.M. Jackson and R.P. Ekins, *Journal of Immunological Methods*, 87:13, 1986) for the desirability and necessity for their assay to be noncompetitive.) In all applications and disclosures of their invention, the removal of excess label-conjugated binders (antibodies) is predicated and is accomplished with the use of "an insoluble material which is attached by a reversible bridge to an analyte derivative or an analyte mimic" (claim 1). The removal of 'analyte-free' labeled binder step is what makes this invention a non-competitive assay. Though Piran and the present application use noncompetitive assay methodologies (a methodology known to those skilled in the art), Piran requires removing the non-reacted labeled antibody; the present application requires that the bound analyte is separated from the analyte solution. Additionally, though Piran teaches the separation of the excess labeled binder through chromatographic methods, this step is known to those skilled in

the art. Interestingly, this kind of chromatographic separation is also taught in Rothschild (column 9, line 22). Accordingly, and after careful assessment of the examiner's comments, Claim 1 has been amended to limit the declared invention to fully incorporate what is novel in the current application.

Rothschild discloses a method in which a detectable moiety (marker) is conjugated to the target (analyte) with photo-labile linkers. In this invention, the targets are cleaved from the substrate with the application of electromagnetic radiation (light) where the substrates are defined as the soluble targets (suitable substrates are identified in column 10, lines 50-64) and detected through their "detectable moieties;" targets are not identified a priori from unknown samples using the method of Rothschild. This definition of substrate is radically different from that provided in the present application (bulk surface upon which specific non-antibody ligands are tethered and upon which detection is accomplished). In the invention disclosed by Rothschild wherein the target substrate is a microorganism (virion, column 10, line 54; cell, column 12, lines 40-47), the photocleavable conjugate is attached to the microbe through covalent bonds (column 12, line 43) and not through binding interactions with photostable tethered ligands. Additionally, in this application of the invention taught by Rothschild, any detected moiety must come from a known (and uncoupled substrate purified) target (column 13, lines 24-26) – knowing the identity of the analyte before the analysis makes taxonomic identification of said target moot.

Piran (column 5, lines 20-23 and column 1, lines 24-25) and Rothschild (column 10, line 54 and column 12, lines 40-47) teach the detection of microorganisms and proteins as is disclosed in Powers (WO 98/49557). However, Powers does not teach the

indicated tether lengths that are to be used for each analyte to be captured. (Powers also doesn't teach the steps of separating the substrate surface from the solution nor washing away non-bound portions of the sample [biological components of the matrix from which the analyte was captured]). It is known to those that work with peptide ligands (illustrated recently by R. Hahn, E. Berger, K. Pflegerl, and A. Jungbauer "Directed Immobilization of Peptide Ligands to Accessible Pore Sites by Conjugation with a Placeholder Molecule, *Anal. Chem.* **2003**, *75*, 543-548) that (1) binding of macromolecules depends more upon the accessibility of the macromolecule to the ligand, and (2) the effects of ligand tether length are realized at much shorter lengths. The following data (shown with circles in the figure below) are provided to illustrate the complexity of the relationship between tether length and binding efficacy when intact microorganisms are involved:



This figure shows the effect of the length of the tether between a surface and a ligand (iron-containing deferoxamine) on the binding of a microorganism (*Klebsiella*

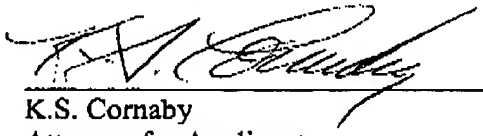
pneumoniae). At shorter tether lengths (<10 Å) binding is superior to that observed at medium lengths (*ca.* 20 Å) and, as is taught in the current application, optimizes at lengths at around 40 Å; longer tether lengths affect affinity little. (These data clearly show that the efficacy of capture of the target microbe is not an easily predictable function of the tether length.) Optimal tether lengths for binding between small, non-antibody based ligands and solubilized free proteins, as disclosed in the claims of the current application, are found at lengths not much greater than six to ten Å. The data from Hahn, *et al.* (shown with squares in the figure above) confirm our disclosure and illustrate the predicted effect of tether length on normalized ligand utilization (μmol target captured/μmol ligand on the surface). We have not observed any improvement in binding of macromolecules (unlike microorganisms) when the tether length exceeds 20 Å; this observation is indirectly confirmed by the fact that there are few commercially available linkers/tethers that exceed 15 Å in length. As there are no obvious physiological structures on the surface of *Klebsiella* spp. to account for the differences between optimal tether lengths of the intact microorganism and its free receptor, it is our position that the indication of the required minimum tether length in the application is not inherently obvious. In summary, required tether lengths depend upon the particle in which the receptor is embedded, NOT on the receptor itself or on the ligand-receptor pair, and this important disclosure is not taught in Powers, et al. Additionally, as pointed out by the examiner, Piran and Rothschild disclose the use of labels (or detectable moieties) that are fluorescent, luminescent and radioactive. However, Piran teaches that these labels are known to those skilled in the art (column 7, lines 15-18) and cites Howanitz, et al. (column 7, lines 24-28) which includes these labels. Claims 10-12 of the current

application teach the use of fluorescent, luminescent and radioactive markers (labels), but limit their use to the specific application as taught in the amended claim 1.

If allowable subject matter is found, Applicants will comply with requirements re Double Patenting later.

It is believed the claims are now in condition for allowance, which action is respectfully requested. Should the Examiner have any questions, he is requested to call Applicants' undersigned attorney collect at (801) 521-3200.

Respectfully submitted,



K.S. Cornaby
Attorney for Applicants
Jones Waldo Holbrook & McDonough PC
170 South Main Street, Suite 1500
Salt Lake City, UT 84101
(801) 521-3200